

OXIDATIVE AND DEGRADATIVE CHANGES OCCURRING IN BABUL LIQUOR

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Heating of the aqueous infusion of babul in hydrolysis tubes for varying periods at 105°C showed that with increase in the period of heating there occurs a gradual degradation of a portion of tannins, a decrease in the total solubles content and elimination of leucocyanidin gallate. Refluxing for 84 hours was found to have the most drastic effect, bringing about the total elimination of leucocyanidin gallate and the two polymeric tan fractions T₁ and T₂, with a slight increase in the nontan content. Exposure to the atmosphere of babul tan liquor and detannised liquor led to pronounced changes, especially during the second and third week of exposure, with considerable loss of tannins and increase in nontan content. The ultimate degradation product in acid and alkaline hydrolysis was gallic acid.

It is known that vegetable tan liquors undergo oxidative and degradative changes. To study the changes that take place when babul liquor is left standing in the tanyard for a long time and to get an idea about the relative stability of the tannin and nontannin components which are of practical significance, the following studies were undertaken with babul aqueous infusion: (a) heating in hydrolysis tubes at 105°C for varying periods, (b) refluxing for a fixed number of hours, (c) exposing to the atmosphere for several weeks and (d) alkaline and acid hydrolysis. These studies were also undertaken with a view to ascertain the stability of the phenolic constituents present in babul as well as to record the various intermediate stages involved in the process of degradation.

Experimental

For all these studies, fresh babul bark, collected from the trees planted on the Institute premises were used. One part of the well crushed bark was leached with three parts of water in the cold for 18 hours. To this leach liquor was added fresh babul bark and the liquor left undisturbed for a period of 24 hours. This was repeated twice; the liquor filtered through cotton wool, centrifuged for 30 minutes at 2000 r.p.m. and filtered. The strength was found to be 20°Bk. This liquor was used in all the cases after necessary dilution wherever necessary.

Chromatographic studies of the aqueous infusion were carried out with 6% acetic acid as solvent for first dimension and *sec.* butanol : acetic acid : water

(14:1:5) as solvent for second dimension¹ using Whatman No. 3 paper and, adopting the ascending technique. The paper was later developed with diazotised para nitroaniline.² A typical chromatogram of the babul aqueous infusion is given in Fig. 1.

(a) *Heating in hydrolysis tubes at 105°C for varying periods*

100 ml. portions of 20°Bk babul liquor were pipetted into three hydrolysis tubes whose ends were then sealed. The tubes were then heated in the air oven for varying periods (25, 75 and 200 hours), the temperature being maintained at 105°C. After the completion of the respective periods, the liquors were filtered, analysed for their tannin and nontannin contents and in addition subjected to chromatographic study.

(b) *Refluxing for fixed number of hours*

250 ml. fresh babul liquor (20°Bk) was taken in a one litre conical flask fitted with a reflux condenser and boiled daily for 7 hours. A portion of the refluxed liquor was withdrawn after 3, 7 and 12 days of refluxing and subjected to regular tannin analysis and chromatographic study.

(c) *Exposing the liquor to atmosphere for several weeks*

The 20°Bk babul infusion was filtered through Whatman No. 11 filter paper and the strength was adjusted to 10°Bk by dilution. The physical and chemical characteristics of the liquor in terms of the contents of tannins, nontannins, salts and acids etc., in addition to its chroma-

tographic behaviour were studied periodically over a period of 5 weeks. In order to arrive at the specific effects brought about by tannins under exposure, parallel experiments were undertaken with detannised liquor. The detannising was carried out by taking 3000 ml. babul liquor (10° Bk) and precipitating the tannins by adding calculated amounts of 10% lead acetate solution so that only trace amounts of tannins were left in the residual liquor. This was considered essential for avoiding as far as possible any excess lead acetate being left in the detannised solution. After addition of lead acetate, the solution was stirred well, centrifuged and the detannised liquor collected. 3000 ml portions of tan liquor and detannised liquor were left exposed to atmosphere in two shallow graduated glass troughs for a period of 5 weeks. After the end of each week, aliquots of both the liquors were withdrawn for regular analysis after making the respective volumes upto the mark,

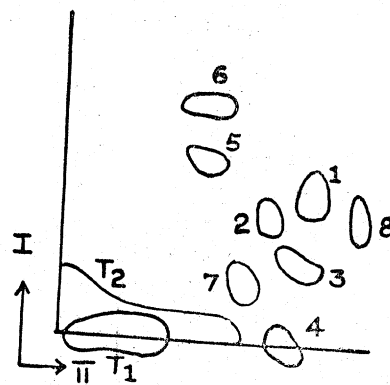


FIG. 1. Chromatograph of aqueous infusion of babul

1. catechin and gallic acid; 2. *epi*-catechin; 3. leucocyanidin gallate; 4. quercetin; 7. dicatchin; 5, 6, 8, unknown; T₁ and T₂—polymeric tannins.

so as to compensate for any loss due to evaporation consequent upon exposure.

(d) Alkaline and acid hydrolyses

The alkaline and acid hydrolyses were carried out by the method of McCalla and Neish³ as modified by Ibrahim and Towers.⁴ Well crushed babul bark was extracted with boiling 80% ethanol by refluxing for 6 hours. The filtered ethanol extract was concentrated under reduced pressure and dried in a vacuum dessicator. Acid hydrolysis was affected by refluxing the ethanol extract (4 g.) with 2N HCl (300 ml.) on a water bath for 2 hours. The acid hydrolysate was then extracted directly with ether in a liquid-liquid extractor in a counter-current fashion for 6 hours. The ether extract was dried under vacuum. Both the residual acid hydrolysate (after ether extraction) and the ether extract were studied chromatographically. Alkaline hydrolysis was carried out with the ethanol extract (4 g) and 4N NaOH (300 ml.), the mixture being allowed to stand for 5 hours at room temperature in nitrogen atmosphere. This was acidified to pH 2.0 using HCl and the ether ex-

traction was carried out as before. The ether extract was washed with 5% NaHCO₃ solution and the washings were acidified and reextracted with ether. The final ether extract was evaporated to dryness and both this residue and the ether insolubles were studied chromatographically as before.

Results and Discussion

(a) Heating in hydrolysis tubes

It can be seen from Table 1 that there is practically no fall in the total solubles content of the liquor up to 75 hours' heating, even though the T/NT ratio has been appreciably altered, the decrease of tannin content being progressive with heating period. After 200 hours' heating, some insolubles were noticed with a corresponding fall in the total solubles content. The tannin content was found to decrease appreciably after 24 hours of heating with further heating, accompanied by gradual increase in the nontannin content. Since this appreciable fall in the tannin content up to 75 hours' heating is not accompanied by any fall in the total solubles content of the liquor, it might be attributed to the phenolic tan-

Table 1
ANALYSIS OF BABUL LIQUOR HEATED AT 105°C FOR VARYING PERIODS

	Hours			
	0	24	75	200
Total solubles (%)	33.54	32.47	32.75	27.56
Tannins (%)	19.39	18.65	15.85	11.90
Nontannins (%)	14.15	13.82	16.90	15.66
T/NT ratio	1.37	1.35	0.94	0.76

Table 2
CHROMATOGRAPHIC DATA OF BABUL LIQUOR HEATED AT 105°C FOR VARYING PERIODS

Period of heating (hours)	Spot numbers			
0	1, 2, 3,	7, 8,	T ₁ , T ₂ ,	4, 5, 6
25	1, 2, 3,	7, 8,	T ₁ , T ₂ ,	X, Y, Z
75	1, 2, 3*,	7, T ₁ ,	T ₂ ,	X, Y, Z
200	1, 2, 3**,	T ₁ ,	T ₂ ,	X, Y, Z

*Intensity faint;

**Intensity very faint

nins, mainly leucocyanidin gallate and its polymer, which are being degraded into leucocyanidin, a polymer of leucocyanidin, and gallic acid, consequent upon heating. The colour of the liquor was found to darken presumably due to polymerisation of tannins brought about by oxidation, consequent on heating, the darkening being progressive with increase in heating period.

From the chromatographic data (Table 2), it was observed that after 25 hours' heating, the chromatogram showed the presence of three extra spots, one of which was identified as pyrogallol by running an authentic sample of pyrogallol in the same solvent systems and comparing the R_f values. Leucocyanidin gallate was found to decrease appreciably after 75 hours' heating and was present in traces only after 200 hours' heating, as was evident from the lack of intensity of the spot. The intensity of the immobile streak was found to become more intense with increased periods of heating. It was also observed that the intensity of gallic acid and pyrogallol was found to increase with increased periods of heating. This confirms the earlier ob-

servation that the appreciable fall in tannin content up to 75 hours' heating might be due to the degradation of leucocyanidin gallate into gallic acid and leucocyanidin as well as polymerisation of the remaining tannin components.

(b) *Refluxing the liquor for fixed number of hours*

There was a very sharp fall in T/NT ratio along with the formation of some insolubles (Table 3). A number of phenolic constituents, including leucocyanidin gallate and polymeric tannin fractions T_1 and T_2 , were completely eliminated (Table 4). Since these three constituents account for almost the entire tannin components present in babul, the marked decrease in the T/NT ratio (0.2 as against 1.37 of the original liquor) can be attributed to the total elimination of these constituents. Condensed tannins, in general, are susceptible to heat in the presence of oxygen, as it facilitates polymerization; hence they would either precipitate out or would lose their tanning potency. This, along with the possibility of leucocyanidin gallate and polymers of leucocyanidin gallate being

Table 3
ANALYSIS OF BABUL LIQUOR REFLUXED FOR
DIFFERENT PERIODS

	Days			
	0	3	7	12
Total solubles	33.54	31.69	30.60	28.64
Tannins	19.39	15.87	10.54	5.36
Nontannins	14.15	15.82	20.06	23.28
T/NT	1.37	1.0	0.52	0.23

Table 4
CHROMATOGRAPHIC DATA OF BABUL LIQUOR
REFLUXED FOR DIFFERENT PERIODS

Period of refluxing (days)	Spot numbers
0	1, 2, 3, 4, 5, 6, 7, 8, T_1 , T_2
3	1, 2, 3*, 7, 8, T_1 , T_2 , X
7	1, 2, 7*, T_1^* , T_2^* , X
12	1, 2, 7*, X

*Intensity very faint

X — Pyrogallol

Table 5

ANALYSIS OF BABUL LIQUOR EXPOSED FOR DIFFERENT PERIODS

	Period of exposure				
	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Total solubles	13.99	11.42	11.69	10.88	10.58
Tannins (g./l.)	8.10	6.65	5.67	4.93	4.48
Nontannins (g./l.)	5.89	4.77	6.02	5.95	6.10
T/NT ratio	1.37	1.39	0.94	0.82	0.73
pH	4.60	5.75	5.90	5.95	5.90
Salts of weak acids (alkalinity of ash method) meq./100 g. T.S.	88.90	93.50	89.60	89.40	89.30
Total salts (sulphated ash method) meq./100 g. T.S.	114.40	121.80	119.90	117.50	112.10
Acidity to pH 5.8 (meq./100 g. T.S.)	143.00	—	—	—	—

T.S. — Total solubles.

degraded into leucocyanidin and polymers of leucocyanidin and gallic acid, might well account for the total elimination of the tannin components of babul.

(c) *Exposing the liquor to atmosphere for several weeks*

Along with the tan liquor, detannised liquor was exposed to atmosphere under identical conditions to study the specific effects caused by tannins under exposure.

Tan liquor: There was a slight decrease in the total solubles content during the second week after which the total solubles content remained more or less the same (Table 5). The nontannin content was found to decrease during the first week but increased appreciably with further increase in the period of exposure. This increase in the nontannin content after two weeks of exposure might be attributed to the breakdown of a portion of phenolic tannins (leucocyanidin gallate into lucocyanidin and gallic acid).

The T/NT was found to decrease with increase in the period of exposure, the fall being appreciable after two weeks exposure. The pH of the liquor was found to increase during second week, thereafter remaining more or less the same with further increase in the period of exposure. Salts of weak acids of the liquor did not show any variation due to exposure. Taken along with the figure

Table 6
CHROMATOGRAPHIC DATA OF BABUL LIQUOR
EXPOSED FOR DIFFERENT PERIODS

Period of exposure (weeks)	Spot numbers
1	1, 2, 3, 4, 5, 6, 7, 8, T ₁ , T ₂
2	1, 2, 3*, 4**, 5*, 6*, 7, 8, T ₁ , T ₂ , X
3	1, 2, 7, 8, T ₁ , T ₂ , X
4	1, 2, 7, 8, T ₁ , T ₂ , X
5	1, 2, 7, 8, T ₁ , T ₂ , X

*Intensity faint

**Intensity very faint

Table 7

ANALYSIS OF DETANNISED BABUL LIQUOR EXPOSED FOR DIFFERENT PERIODS

	Period of exposure				
	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Total solubles (g./l.)	7.73	7.12	5.81	5.51	5.34
Tannins (g./l.)	1.76	1.91	0.90	0.69	0.64
Nontannins (g./l.)	5.97	5.21	4.91	4.82	4.70
T/NT ratio	0.29	0.36	0.19	0.14	0.14
pH	4.30	4.80	5.10	5.20	5.35
Salts of weak acids (alkalinity of ash method) meq./100 g. T.S.	149.40	136.20	164.90	149.30	146.40
Total salts (sulphated ash method) meq./100 g. T.S.	239.10	246.90	241.80	257.40	249.90
Acidity to pH 5.8 (meq./100 g. T.S.)	794.50	436.00	216.70	141.30	65.60

for total salts, it would appear that most of the salts present in babul are salts of weak acids; this is in conformity with the earlier observation of Burton and Barat.⁵

From the chromatographic data (Table 6), it was observed that leucocyanidin gallate and quercetin were among the constituents that were eliminated on exposure during the third week itself. Pyrogallol was identified as a new constituent formed on exposure during the second week and its presence was observed throughout the entire period of exposure. The two polymeric tannin fractions T_1 and T_2 were found to decrease with increase in the period of exposure.

Detannised liquor: In the case of detannised liquor, the total solubles content was not found to decrease significantly (Table 7). The amount of precipitation was practically negligible. The T/NT ratio recorded an apprecia-

ble increase during the first week of exposure with a pronounced decrease after exposure for two weeks. A similar increase in T/NT was observed in the case of tan liquor also during the second week's exposure. In the case of detannised liquor, a decrease in total solubles content followed by a slight increase in the tan content and considerable decrease in nontan content was observed during the first week's exposure. Hence, it is difficult to explain whether the increase in T/NT ratio during first week is due to the conversion of some phenolic nontannins into tannins or elimination as insolubles. It might appear from this that it would be an advantage to expose babul liquor for a week prior to use. But, in view of the sharp decrease in tannins during second week of exposure, it would be advisable to use fresh liquor.

The pH of the detannised liquor was found to increase progressively with increase in the period of exposure to at-

Table 8
CHROMATOGRAPHIC DATA OF DETANNISED BABUL
LIQUOR EXPOSED FOR DIFFERENT PERIODS

Period of exposure (weeks)	Spot numbers
1	1, 2, 7, 8
2	1, 2, 7, 8, X
3	1, 2, 7, 8, X
4	1, 2, 7, 8, X
5	1, 2, 7, 8, X

mosphere. This might be due either to the formation of neutral salts or to the evaporation of the residual acetic acid in the solution derived from precipitation of the tannins with lead acetate. But taking into account the very sharp decrease in the total acidity as determined by titration to pH 5.8, the increase in pH is more likely to be due to the loss of acetic acid by evaporation.

The chromatograms did not show any variation except for the presence of pyrogallol after the initial stages of exposure.

(d) Alkaline and acid hydrolyses

The ether solubles of the hydrolysate in both cases when studied chromatographically revealed the presence of gallic acid and one new unknown constituent (R_f 0.95, 0.03) (Table 9). The ether insolubles of the hydrolysate showed, in addition to these spots, a faint streak corresponding to the position of immobile components T_1 and T_2 . The presence of gallic acid in the ether insolubles would mean that the ether extrac-

Table 9
CHROMATOGRAPHIC DATA OF ACID AND ALKALINE
HYDROLYSATE OF BABUL

Spot numbers	
Acid hydrolysate	
Ether solubles	1, N
Ether insolubles	1, N, T_1^{**}
Alkaline hydrolysate	
Ether solubles	1, N
Ether insolubles	1, N, T_1^{**}

** Very faint. R_f value of N= 0.95, 0.03.

tion is perhaps incomplete. The new constituent must be a degradation product resulting from hydrolysis since it was absent in the original liquor. From the intensity of the spots, however it appears that gallic acid accounted for a major portion of the degradation product, mainly from leucocyanidin gallate and polymers of leucocyanidin gallate.

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